

Please cancel claims 1-17, 24, 26-28 and 31 and replace with new claims 32-47.

What is claimed is:

1-17 (cancelled)

18. (withdrawn) A nucleic acid comprising a DNA sequence that substantially corresponds to SEQ ID NO:1 wherein at least one mutation has been introduced in the sequence corresponding to the β -bridge.

19. (withdrawn) The nucleic acid according to claim 18, wherein the mutation occurs at a site which encodes the PA in the β -bridge.

20. (withdrawn) The nucleic acid according to claim 19, wherein the mutation at the site that encodes PA is a substitution or deletion such that the substituted nucleic acid encodes a single amino acid, a dipeptide, a tripeptide and a tetrapeptide.

21. (withdrawn) A nucleic acid according to claim 17, wherein the at least one mutation results in an amino acid change selected from PA/A, PA/AA, PA/PGA, PA/PAPA, Δ PA, PA/K, PA/G, PA/D and PA/P.

22. (withdrawn) A vector encoding the nucleic acid of any of claim 18 through 21.

23. (withdrawn) A host cell containing a vector of claim 22.

24 (cancelled)

25. (withdrawn) A method for modifying enzyme catalytic activity, comprising:

(a) selecting an enzyme having two catalytic centers connected by a β -bridge, the catalytic centers being located at reciprocal stereo-geometric positions in the enzyme;

(b) changing the reciprocal stereo-geometric position of the two catalytic centers by introducing a mutation into the β -bridge; and

(c) modifying the catalytic activity of the enzyme.

26-28 (cancelled)

29. (withdrawn) A method of forming a shotgun cloning library, comprising

(a) incubating a modified DNA cleaving enzyme according to claim 1 with a DNA to form non-sequence specific cleavage fragments of the DNA that are ligatable; the ligatable DNA being capable of insertion into a vector for cloning in a host cell; and

(b) forming the shotgun cloning library.

30. (withdrawn) A method for mapping nicks in a duplex DNA, comprising;

(a) incubating a modified DNA cleaving enzyme according to claim 1 with the duplex DNA in a manganese-containing buffer;

(b) permitting nicking to occur across from a pre-existing nick site to form fragments of the duplex DNA with single strand overhangs; and

(c) mapping the nicks in the DNA.

31. (cancelled)

32. (new) A composition, comprising:

a recombinant protein having DNA cleavage activity, the protein further characterized by two catalytic centers separated by a β -bridge and having at least 35% amino acid sequence identity with SEQ ID NO:12 for a T7 Endo I, wherein the recombinant protein when compared with T7 Endo I has:

- (a) reduced toxicity for an *E. coli* host cell; and
- (b) an amino acid sequence in the beta bridge that differs by at least one amino acid from SEQ ID NO:12.

33. (new) The composition according to claim 32, wherein the DNA cleavage activity results in a product, wherein the product is a DNA duplex with a single strand overhang of less than 11 nucleotides.

34. (new) The composition according to claim 32, wherein the DNA cleavage activity comprises at least one of the group consisting of: cleavage at a cruciform structure on DNA; non-sequence-specific nicking; nicking opposite a pre-existing nick site; non-sequence-specific DNA cleavage; and cleavage of DNA at a site flanking a mismatch base pair.

35. (new) The composition according to claim 32, wherein the DNA cleavage activity further comprises cleaving at a mismatch in a DNA duplex where the mismatch can be any of an A, T, G or C bases.

36. (new) The composition according to claim 32, further comprising:
(c) greater DNA cleavage activity of the recombinant protein in a

manganese-containing buffer compared with DNA cleavage activity of T7 Endo I in the manganese-containing buffer.

37. (new) The composition according to claim 36, wherein one or more DNA cleavage activities are selected from the group consisting of: cleavage of a cruciform DNA; nicking opposite a preexisting nick site; nicking next to a mismatch in the DNA resulting in double strand cleavage; and non-specific nuclease activity.

38. (new) The composition according to claim 32, further comprising:
(c) reduced DNA cleavage activity of the recombinant protein in a magnesium-containing buffer compared with DNA cleavage activity of T7 Endo I in the magnesium-containing buffer.

39. (new) The composition according to claim 38, wherein one or more DNA cleavage activities are selected from the group consisting of: nicking opposite a preexisting nick site; nicking next to a mismatch in the DNA resulting in double strand cleavage; and non-specific nuclease activity.

40. (new) The composition according to claim 32, wherein the recombinant protein is derived from a T7-like endonuclease selected from the group consisting of: gene 3 (Enterobacteria phage T7), T7 endodeoxyribonuclease I, *Yersinia pestis* phage phiA1122 endonuclease, Phage PhiYe03-12 endonuclease, Phage T3 endodeoxyribonuclease, *Pseudomonas* phage gh-1 endonuclease, *Pseudomonas putida* KT2440; Endodeoxyribonuclease I; and *Roseophage* S101 RP endonuclease I.

41. (new) The composition according to claim 32, wherein the difference of at least one amino acid in the β -bridge is a substitution or a deletion at a site corresponding to a Pro-Ala (PA) dipeptide in the β -bridge of SEQ ID NO:12.

42. (new) The composition according to claim 41, wherein the substitution is a single amino acid substitution, a dipeptide substitution, a tripeptide substitution or a tetrapeptide substitution at the PA; or a deletion of PA, Pro(P) and Ala(A).

43. (new) The composition according to claim 42, wherein the PA dipeptide is substituted with an Ala (A), a Lys (K), a Gly (G), an Asp (D) or a Pro (P), an Ala-Ala (AA) dipeptide, an Ala-Gly-Ala (AGA) tripeptide, a Pro-Ala-Pro-Ala (PAPA) peptide, or a deletion of PA.

44. (new) A kit containing at least one of: the composition of claim 41; an isolated nucleic acid comprising the composition of claim 1; a vector comprising the nucleic acid; or a host cell comprising the vector.

45. (new) A method of determining whether a DNA substrate has a single nucleotide polymorphism (SNP), comprising:

(a) contacting the DNA substrate with the composition according to claim 32; and

(b) determining from the cleavage product whether the DNA substrate has the SNP.

46. (new) The method according to claim 45, further comprising: identifying which nucleotide forms the SNP.

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Serial No.: 10/585,964
Date Filed: July 13, 2006
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47. (new) The method according to claim 45, further comprising:
identifying the location of the SNP.